Does venous blood gas analysis provide accurate estimates of hemoglobin oxygen affinity?

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Abstract: Alterations in hemoglobin oxygen affinity can be detected by exposing blood to different PO(2) and recording oxygen saturation, a method termed tonometry. It is the gold standard to measure the PO(2) associated with 50 % oxygen saturation, the index used to quantify oxygen affinity (P(50)Tono). P(50)Tono is used in the evaluation of patients with erythrocytosis suspected to have hemoglobin with abnormal oxygen affinity. Since tonometry is labor intensive and not generally available, we investigated whether accurate estimates of P(50) could also be obtained by venous blood gas analysis, co-oximetry, and standard equations (P(50)Ven). In 50 patients referred for evaluation of erythrocytosis, pH, PO(2), and oxygen saturation were measured in venous blood to estimate P(50)Ven; P(50)Tono was measured for comparison. Agreement among P(50)Ven and P(50)Tono was evaluated (Bland-Altman analysis). Mean P(50)Tono was 25.8 (range 17.4-34.1) mmHg. The mean difference (bias) of P(50)Tono-P(50)Ven was 0.5 mmHg; limits of agreement (95 % confidence limits) were -5.2 to +6.1 mmHg. The sensitivity and specificity of P(50)Ven to identify the 25 patients with P(50)Tono outside the normal range of 22.9-26.8 mmHg were 5 and 77 %, respectively. We conclude that estimates of P(50) based on venous blood gas analysis and standard equations have a low bias compared to tonometry. However, the precision of P(50)Ven is not sufficiently high to replace P(50)Tono in the evaluation of individual patients with suspected disturbances of hemoglobin oxygen affinity.

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Does venous blood gas analysis provide accurate estimates of hemoglobin oxygen affinity?

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Abstract

Background: Alterations in hemoglobin oxygen affinity can be detected by exposing blood to different PO2 and recording oxygen saturation, a method termed tonometry. It is the gold standard to measure the PO2 associated with 50% oxygen saturation, the index used to quantify oxygen affinity (P50Tono). P50Tono is used in the evaluation of patients with erythrocytosis suspected to have a hemoglobin with abnormal oxygen affinity. Since tonometry is labor intensive and not generally available we investigated whether accurate estimates of P50 could also be obtained by venous blood gas analysis, co-oximetry and standard equations (P50Ven).

Methods: In 50 patients referred for evaluation of erythrocytosis, pH, pO_2 and oxygen saturation were measured in venous blood to estimate P50Ven; P50Tono was measured for comparison. Agreement among P50Ven and P50Tono was evaluated (Bland-Altman analysis).

Results: Mean P50Tono was 25.8 (range 17.4-34.1) mmHg. The mean difference (bias) of P50Tono-P50Ven was 0.5 mmHg, limits of agreement (95% confidence limits) were -5.2 to +6.1 mmHg. The sensitivity and specificity of P50Ven to identify the 25 patients with P50Tono outside the normal range of 22.9-26.8 mmHg were 5% and 77% respectively.

Conclusions: Estimates of P50 based on venous blood gas analysis and standard equations have a low bias compared to tonometry. However the precision of P50v is not sufficiently high to replace P50Tono in the evaluation of individual patients with suspected disturbances of hemoglobin oxygen affinity.

Keywords: erythrocytosis, diagnosis, hemoglobin, oxygen affinity, polycythemia

Introduction

The causes of erythrocytosis, i.e., an increased erythrocyte mass, include either a decreased plasma volume (relative erythrocytosis) or an increase in the erythrocyte mass (absolute erythrocytosis) as determined by radionucleotide techniques. Absolute erythrocytosis may be primary: due to myeloproliferative neoplasia (polycythemia vera), congenital due to erythropoietin-receptor mutations, or secondary: due to various causes including cardio-pulmonary or renal disease, congenital due to defects of the oxygen sensing pathway (*VHL*, *PHD2* or *HIF*-2α gene mutations) or other rare congenital defects like hemoglobin variants or bisphosphoglycerate mutase deficiency (table 1). Guideline for the investigation of erythrocytosis [1] [2] suggest to search for polycythemia vera and common causes by history including the use of medication, clinical examination, full blood count, renal and liver function tests, serum ferritin, vitamin B12, and erythropoietin measurement, arterial blood gas analysis, chest radiography and abdominal ultrasonography. If the etiology of the erythrocytosis remains unclear a more specialized stage 2 evaluation includes whole body CT-scan, bone marrow examination, cytogenetics of the bone marrow aspirate, high performance liquid chromatography of the hemoglobin and determination of the oxygen-hemoglobin dissociation curve. It allows to detect hemoglobin variants with altered oxygen affinity as rare causes of erythrocytosis [3][4]. The hemoglobin oxygen affinity is quantified during tonometry by exposing blood to changing partial pressures of oxygen (PO2) while continuously recording oxygen saturation. The P50, i.e., the PO2 at which 50% of the hemoglobin is saturated with oxygen, is used as the numeric value characterizing hemoglobin oxygen affinity (figure 1). Unfortunately, tonometry is time consuming and requires expensive equipment. The technique is therefore not widely available. Estimation of the P50 from venous blood gas analysis and co-oxymetry using standard equations according to Severinghaus [5] has been suggested as a simpler alternative to tonometry. However, this approach to determination of the P50 has not been well validated [6] [7]. The purpose of the current study is therefore to evaluate the accuracy of P50 estimated from venous blood gas analysis in comparison to tonometry, the gold standard for measurement of P50, in patients referred for evaluation of erythrocytosis.

Methods

Patients

Data from all patients referred to the Hematology and Pulmonary Division, University Hospital of Zurich, from 2007 to 2009 for arterial and venous blood gas analysis and tonometry as part of the diagnostic evaluation of erythrocytosis were included. The protocol was in accordance with the Declaration of Helsinki and approved by the Institutional Ethics Committee of the University Hospital of Zurich.

Measurements

Blood analysis

Venous and arterial blood samples were obtained from a cubital vein and radial artery, respectively, and analysed immediately. Blood gas analysis and co-oximetry were performed by an ABL (700 Series, Radiometer Copenhagen) device. P50 was measured by tonometry (P50Tono) (HEMOX-analyser, model B, TCS, Medical Products Division, Southampton, PA). The tonometer measures hemoglobin fractions by two-wavelength spectrophotometry, and PO2 by a Clark electrode during exposure of the blood sample to increasing O2 fractions from 0.21 to 1.0. Validation studies and the normal range for the P50 measured with this device (22.9-26.8 mmHg) have been published [8]. P50 was also calculated from the oxygen saturation and PO2 measured in the venous blood sample incorporating pH adjustments according to Severinghaus (P50Ven). [5]:

$P_{50} = 26.7 \times P_{O2}(obs)/P_{O2}(std)$ (1)

Where $P_{O2}(obs)$, pH 7.4 $= P_{O2} \times e^{1,1(pH - 7.4)}$ (2)

And $P_{O2}(std)$: $\exp(0.385 \ln(S^{-1} - 1)^{-1} + 3.32 - (72S)^{-1} - S^6/6)$ (3)

The normal range for the P50 calculated with this formula is 25.3-30.7 mmHg [9].

Clinical diagnosis

A detailed medical history, clinical examination and laboratory tests were obtained in all patients according to published guidelines for evaluation of erythrocytosis. [1] [2]

Data analysis and statistics

Data are summarized by counts, means and SD. The accuracy of the P50 calculated from the venous blood using the Severinghaus equation was compared to corresponding values measured by tonometry by a Bland-Altman

analysis [10]. Diagnostic accuracy of P50Ven was also determined in terms of sensitivity and specificity to detect an abnormal P50Tono.

Results

Data from 50 patients (13 women) with mean age 46.1 y, SD 15.5, and a range from 20 to 76 y were available. Their clinical diagnoses along with the number of patients with a P50Tono outside the normal range of 22.9 to 26.8 mmHg are listed in table 2. In 14 patients the cause of erythrocytosis could not be definitively determined despite extensive evaluations. Tonometry revealed a mean P50Tono of 25.8 mmHg (range 17.4 mmHg to 34.1 mmHg) with 25 of the 50 values outside the normal range. Only seven of the 25 abnormal results were below the lower limit of 22.9 mmHg, in one of these patients abnormal hemoglobin with high oxygen affinity could be identified (Hemoglobin Cutlerville). The majority of patients with myeloproliferative disorder showed an elevated p50Tono (>26.8mmHg), probably due to secondary regulatory mechanisms.

The corresponding P50Ven was 26.3 mmHg (range 20.9 mmHg to 29.4 mmHg). Figure 2 shows the Bland-Altman analysis evaluating the accuracy of P50Ven compared to P50Tono. The bias was +0.5 mmHg with limits of agreement (95% confidence interval) from -5.2 mmHg to +6.1 mmHg. Table 3 shows the diagnostic performance of the P50Ven compared to P50Tono. The sensitivity was 5%, the specificity was 77% and the positive and the negative predictive values were 12% and 57%, respectively.

Discussion

The current study is the largest comparison of P50 estimated by blood gas analysis and co-oxymetry of venous blood using standard equations with tonometry, the gold standard for P50, in patients with erythrocytosis. We found that despite a negligible bias, the precision of the values of P50Ven was not sufficiently high to allow their use as substitutes for P50Tono in the clinical evaluation of polycythemia in individual patients.

Unfortunately, determination of P50 by tonometry is not feasible in many institutions because it is labour intensive, time consuming and requires specialized equipment. Replacement of this technique by a simpler method based on venous blood gas analysis would therefore be desirable. However, P50Ven has not been well established as a means to assess hemoglobin oxygen affinity, possibly due to the lack of appropriate validation. We identified only two studies involving less than 30 patients in whom P50Ven was evaluated in comparison to tonometry. The first one has been published by Kohzuki et al. [7]. In this study the P50Ven of 25 normal Japanese adults has been compared to the P50 obtained by microtonometry. With this technique 6 discrete points on the dissociation curve at different PO2 were determined by a tonometer instead of the complete dissociation curve recorded in the current study. The mean ±SD deviation of the p50 derived from venous blood and the 6 point technique in the cited study was 0.4±2.5 Torr (at pH 7.4, PCO2 40 Torr and 37°C); similar results were obtained for pH 7.2 and 7.6. In the second study by Aberman et al. [6] the P50Ven at various SO2 (between 20% and 90%) were determined in 135 blood samples from 21 healthy non-smokers and in 8 patients. The standard deviation of P50Ven on the same sample of blood at different SO2 was ±1.0 Torr. A validation of the P50Ven comparison to P50Tono has not been performed in this study. Based on their results, the authors of both studies suggested that P50Ven could serve as a simple surrogate for assessment of the hemoglobin oxygen affinity. However, our data obtained in a larger group of 50 patients do not support these expectations because of the imprecision of P50Ven values in comparison to tonometry. The scatter of P50Ven resulted in a low diagnostic accuracy in detecting patients with an abnormal P50Tono with positive and negative predictive values of only 12% and 57%, respectively which is inappropriate for clinical use.

Based on our data we are unable to differentiate whether the imprecision of P50Ven was mainly related to instrument variability or to physiological reasons. However, the measurement techniques used for blood gas analysis and co-oximetry are the same as for tonomotrey and we therefore speculate that deviations in the shape of the individual hemoglobin dissociation curve from the standard curve explains some of the discrepancy between P50Ven and P50Tono. The narrow normal range of P50 (3.9 mmHg, i.e., from 22.9 to 26.8 mmHg) together with the only slight deviation of P50 that are associated with abnormal hemoglobin variants (see

example in figure 1) suggest that measurement of P50 should be performed with a greater precision that what can be expected from P50Ven. According to a study by Guarnone et al. [8], the P50Tono measured by the tonometer used in the current study has relatively small intra-assay variability with a standard deviation of 0.39 mmHg suggesting that relatively small deviations of P50 from the normal range can be detected.
Guidelines for investigation of erythrocytosis by McMullin et al. [1][2] suggest that a genetic analysis of a potential hemoglobin variant should only be performed if other, more common causes of erythrocytosis have been evaluated. Our study indicates that determination of P50 by tonometry but not P50Ven may help to further guide clinical investigations directed at identifying rare causes of polycythemia. This is also emphasized by a recent study by Rumi et al. [11], where tonometry, besides other clinical tests, illustrated the utility of P50-measurement in the diagnostic process of isolated erythrocytosis in 102 patients with erythrocytosis.

Conflict of interest

The authors declare that they have no conflit of interest.

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Figure legends

Figure 1. Standard oxygen dissociation curve according to the Severinghaus equation. The arterial PO_2 (10.9 kPa), the venous PO_2 (3.8 kPa), and the P50 measured by tonometry (2.3 kPa) in a patient with erythrocytosis due to a high-affinity hemoglobin variant (heterozygous mutation for hemoglobin Cutlerville) are indicated. The P50 estimated from venous blood gas analysis and co-oximetry (2.8 kPa) has been calculated by linear extrapolation of the venous PO_2 to a saturation of 50%. The values of P50 are situated to the left of the standard curve indicating an increased oxygen affinity.

Figure 2. Bland-Altman analysis of P50 estimated from a venous blood gas analysis using the Severinghaus equation vs. corresponding values measured by tonometry. Upper panel: identity plot; lower panel differences vs. mean values. Different symbols reflect the etiology of the erythrocytosis (myeloproliferative vs. other etiologies).

Table 1. Differential diagnosis of erythrocytosis

Primary causes of erythrocytosis		
Congenital:	Erythropoietin receptor mutations	
Acquired:	Polycythemia vera	
Secondary causes of erythrocytosis		
Congenital:	Defects of the oxygen sensing pathway:	VHL gene mutations
		PHD2 mutations
		HIF-2α mutations
	Other congenital defects:	High oxygen-affinity hemoglobin
		Bisphosphoglycerate mutase deficiency
Acquired:	Central hypoxia:	Smoker's erythrocytosis
		Chronic lung disease
		Hypoventilation syndromes including obstructive sleep apnea
		Right-to-left cardiopulmonary vascular shunts
		High altitude
		Carbon monoxide poisoning
	Local hypoxia:	Renal artery stenosis
		Hydronephrosis
		Renal cysts (polycystic kidney disease)
		Post-renal transplant erythrocytosis
		End-stage renal disease
	Pathologic EPO production	Cerebellar hemangioblastoma
		Meingeoma
		Parathyroid carcinoma / adenoma
		Hepatocellular carcinoma
		Renal cell cancer
		Pheochromocytoma
		Uterine leiomyoma
	Drug associated	Erythropoietin administration
		Androgen administration
Idiopathic erythrocytosis		

Table 2. Diagnoses of patients with normal and abnormal P50 by tonometry*

Diagnosis	n patients	P50 by tonometry, mmHg			Suspected etiology of abnormal P50
		low <22.9	Normal range 22.9-26.8	High >26.8	
Myeloproliferative neoplasia	15	2	6	7	JAK-2 mutation was positive in 7, negative in 2, and not analyzed in 6 patients
Smokers erythrocytosis	11	0	8	3	elevated COHb >3%
Relative erythrocytosis	9	1	6	2	Gaisböck-Syndrome
High affinity hemobglobin	1	1	0	0	Hemoglobin Cutlerville
Post renal transplant erythrocytosis	1	0	0	1	Erythropoietin elevation
Cardiovascular disease	1	0	0	1	Congenital Pulmonary stenosis
Pulmonary disease	1	0	1	0	COPD
Hereditary spherocytosis	1	0	1	0	Erythrocyte membrane disorder
Idiopathic/unclear	14	3	6	5	etiology not identified
Total*	54	7	28	19	

* The total number exceeds 50 because 4 smokers had a combined etiology of erythrocytosis (1 patient had chronic obstructive pulmonary disease (COPD), 1 had a myeloproliferative neoplasia and 2 had Gaisböck syndrome)

Table 3. Diagnostic performance of P50 estimated from venous blood gas analysis

		P50 calculated from venous blood gas analysis (P50Ven)	
		Abnormal	normal
P50 measured by tonometry (P50Tono)	Abnormal	1 (low)	24 (P50Tono low in 6, high in 18)
	Normal	7 (P50Ven low)	18

Figure 1

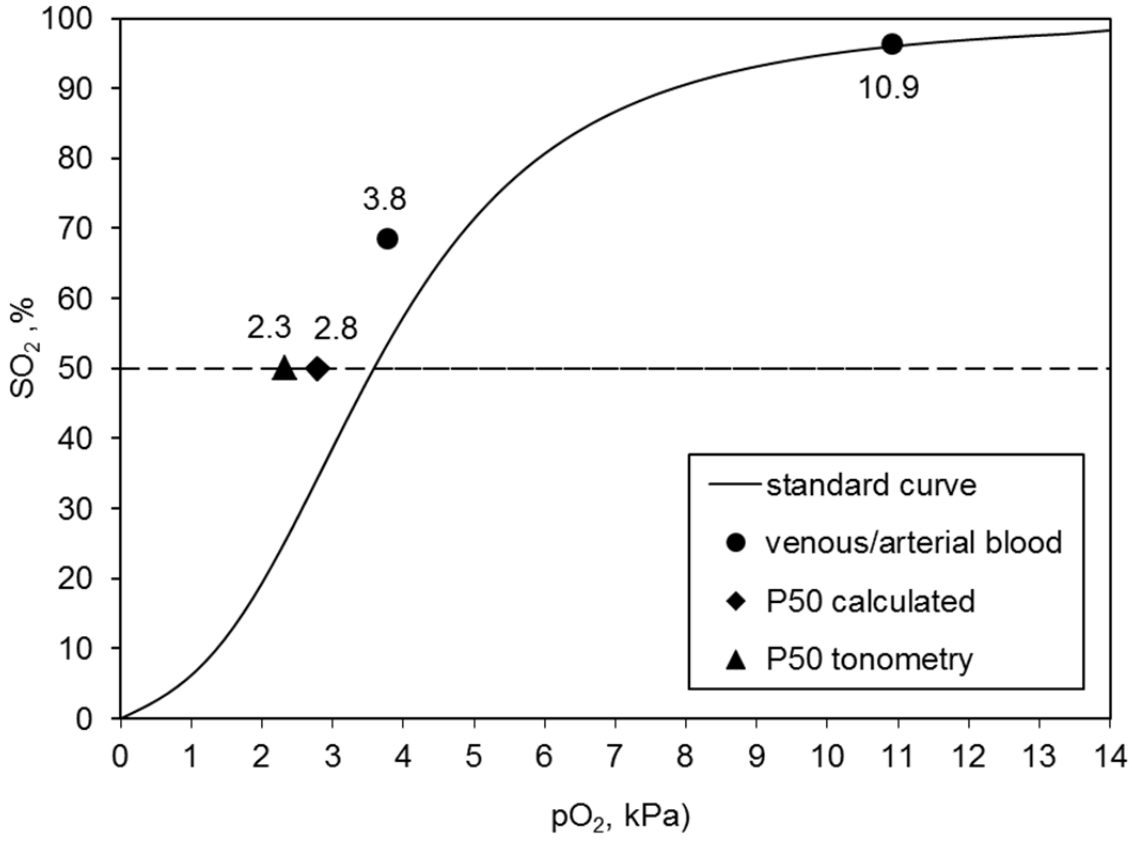
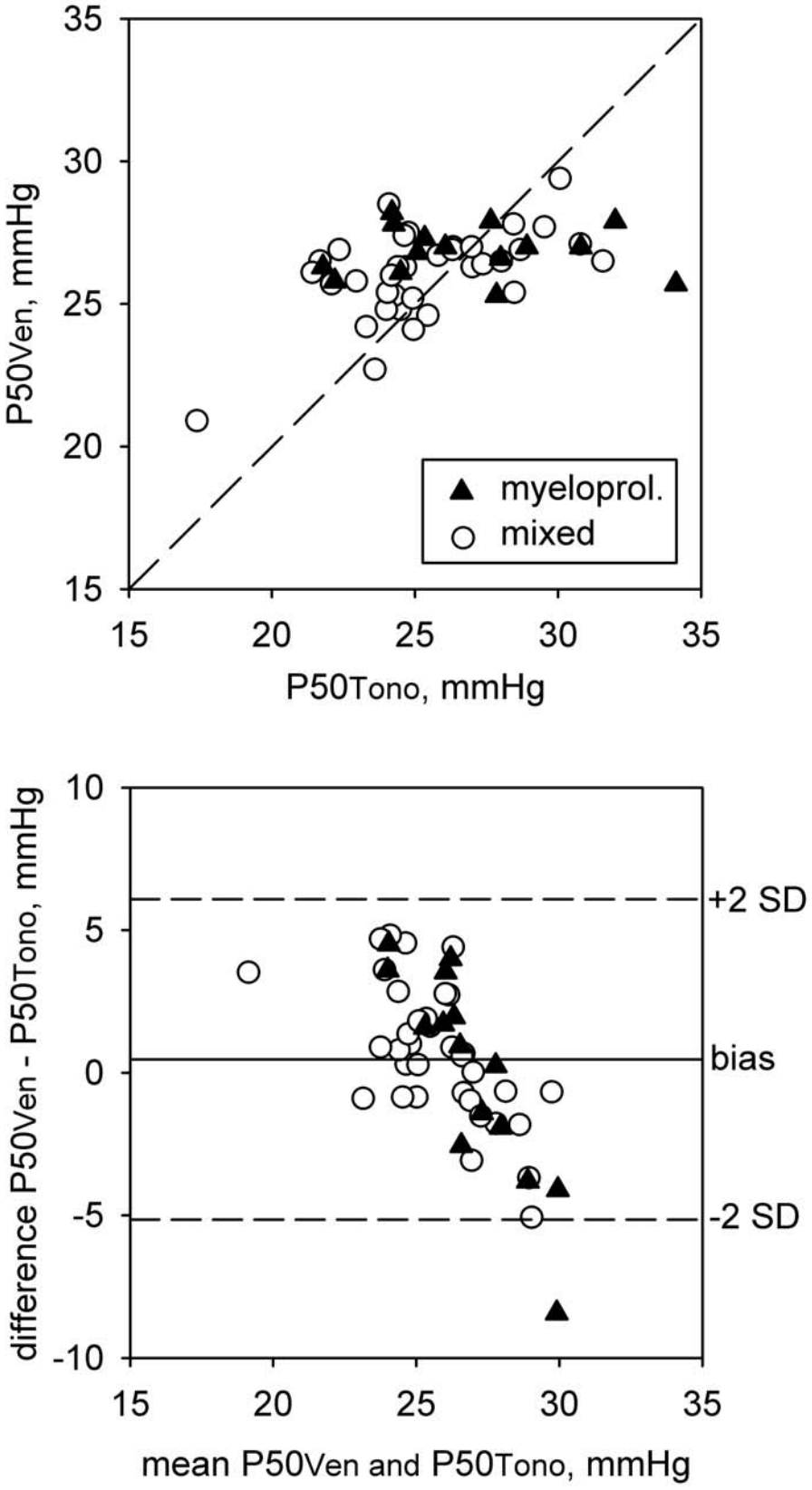

100
90
80
70
60
50
40
30
20
10
0
SO_2, %
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14
pO_2, kPa)
10.9
3.8
2.3
2.8
standard curve
venous/arterial blood
P50 calculated
P50 tonometry

Figure 2


35
30
25
20
15
P50Ven, mmHg
myeloprol.
mixed
15
20
25
30
35
P50Tono, mmHg
10
5
0
-5
-10
difference P50Ven - P50Tono, mmHg
+2 SD
bias
-2 SD
15
20
25
30
35
mean P50Ven and P50Tono, mmHg